1297: NATRONIELLA SULFIDIGENA MEDIUM

$NaHCO_3$	25.00	g
Na_2CO_3	140.00	g
NaCl	15.00	g
K_2HPO_4	1.00	g
NH_4Cl	0.20	g
$MgCl_2$ x 6 H_2O	0.20	g
Yeast extract	0.20	g
Trace elements solution (Pfennig & Lippert,1966)	1.00	ml
Selenite-tungstate solution	1.00	ml
Glycerol	2.00	g
Wolin's vitamin solution (10x)	1.00	ml
Sulfur (powdered)	2.00	g
Na_2S x 9 H_2O	0.25	g
Distilled water	1000.00	ml

Dissolve hydrogen carbonate, carbonate, sodium chloride, and hydrogen phosphate, then sparge medium with 100% N_2 gas for 30 - 45 min to make it anoxic. Dispense solution under the same gas atmosphere into anoxic Hungate-type tubes or serum vials and autoclave. After sterilization add ammonium chloride, magnesium chloride, yeast extract, trace elements, glycerol, vitamins, and sulfide from a sterile anoxic stock solution prepared under 100% N_2 gas. The vitamin solution should be sterilized by filtration. Sulfur is sterilized by steaming for 3 hours on each of 3 successive days (see medium 35) and added aseptically to the sterile medium as powder while retaining anoxic conditions. Adjust the pH of the complete medium to 9.8 - 10.0, if necessary.

Trace elements solution (Pfennig & Lippert,1966) (from medium 1369)

EDTA	5.00	g
$FeSO_4$ x 7 H_2O	2.20	g
$ZnSO_4$ x 7 H_2O	0.10	g
$MnCl_2$ x 4 H_2O	0.03	g
H_3BO_3	0.03	g
$CoCl_2$ x 6 H_2O	0.20	g
$CuCl_2$ x 2 H_2O	0.03	g
$NiCl_2$ x 6 H_2O	0.03	g
Na_2MoO_4 x 2 H_2O	0.03	g
Distilled water	1000.00	ml

pH 3.0-4.0

Selenite-tungstate solution (from medium 385)

NaOH	0.50	g
Na_2SeO_3 x 5 H_2O	3.00	mg
Na_2WO_4 x 2 H_2O	4.00	mg
Distilled water	1000.00	ml

Wolin's vitamin solution (10x) (from medium 120)

Biotin	20.00	mg
Folic acid	20.00	mg
Pyridoxine hydrochloride	100.00	mg
Thiamine HCl	50.00	mg
Riboflavin	50.00	mg
Nicotinic acid	50.00	mg
Calcium D-(+)-pantothenate	50.00	mg
Vitamin B_{12}	1.00	mg
p-Aminobenzoic acid	50.00	mg
(DL)-alpha-Lipoic acid	50.00	mg
Distilled water	1000.00	ml